Supporting Information

Electrostatic interactions as mediators in the allosteric activation of PKA RIα

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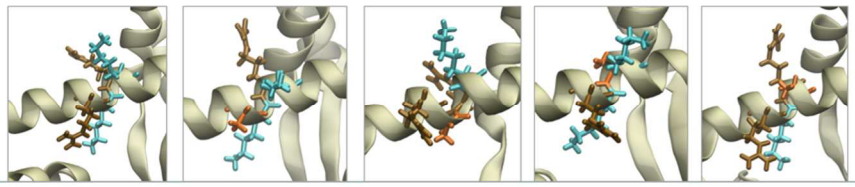
Table S1. Details of the simulated systems. Arginines shown in cyan, lysines in ochre and mutated residues in orange



System	wildtype	R239A	K240A	R241A	K242A
# of atoms	82,561	82,569	82,568	82,581	82,571
# of independent runs	5	5	5	5	5
Simulation length (μs)	1	1	1	1	1
Total sim. time (μs)	5	5	5	5	5

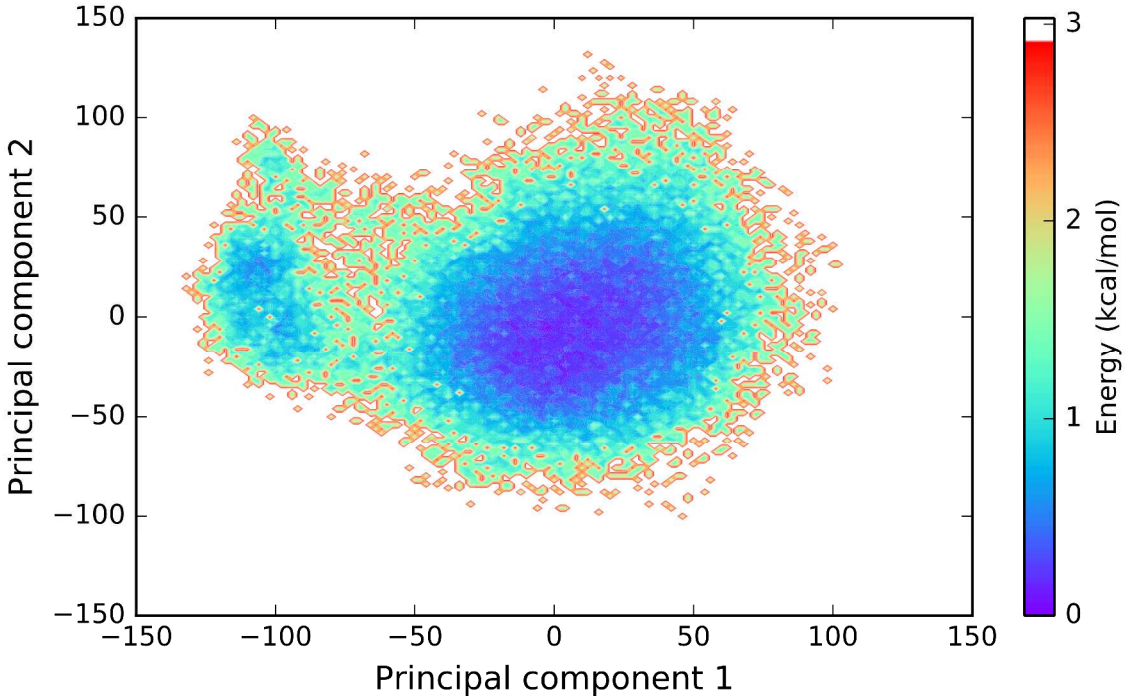


Figure S1. Principal components analysis of wildtype trajectory.

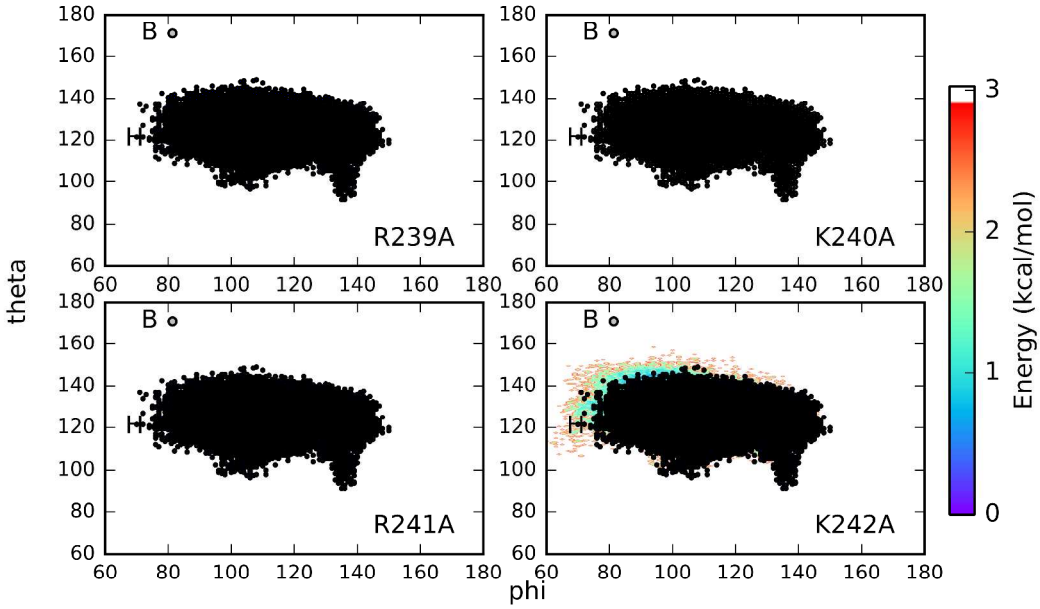


Figure S2. Mutants free energy landscape in terms of spherical angles overlaid in the wildtype sampling conformation (gray outline).

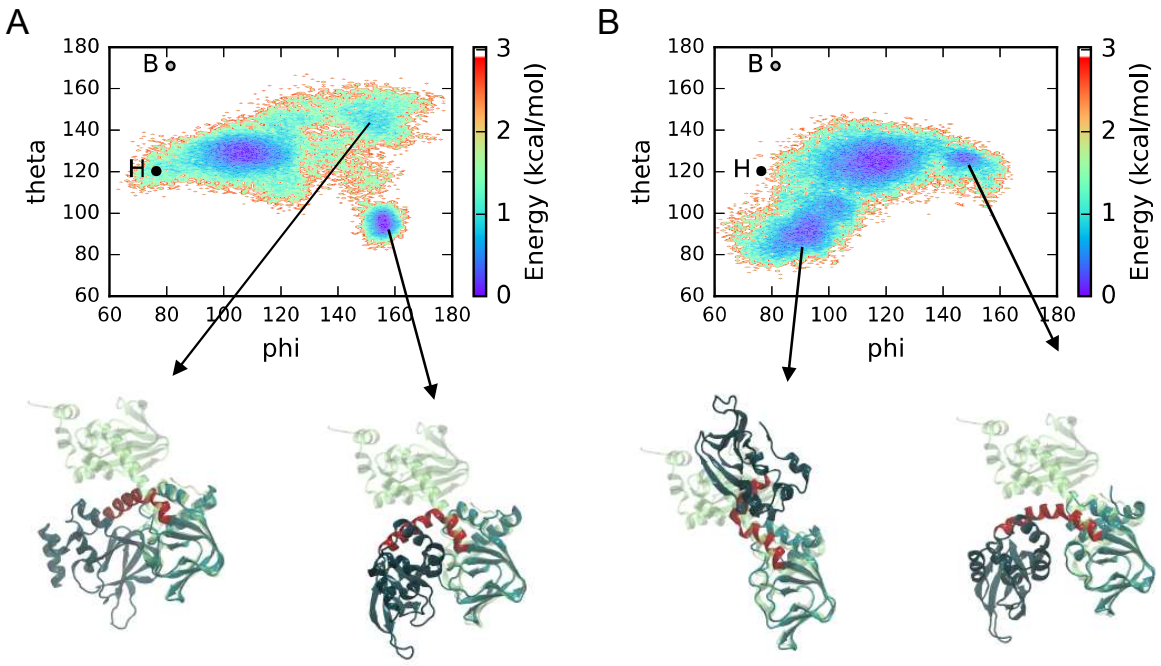


Figure S3. Structural assignment to the free energy landscapes of (a) R239A and (b) R241A. CBD-A shown in cyan, B/C helix in red, CBD-B in dark green and the H conformation in light green.

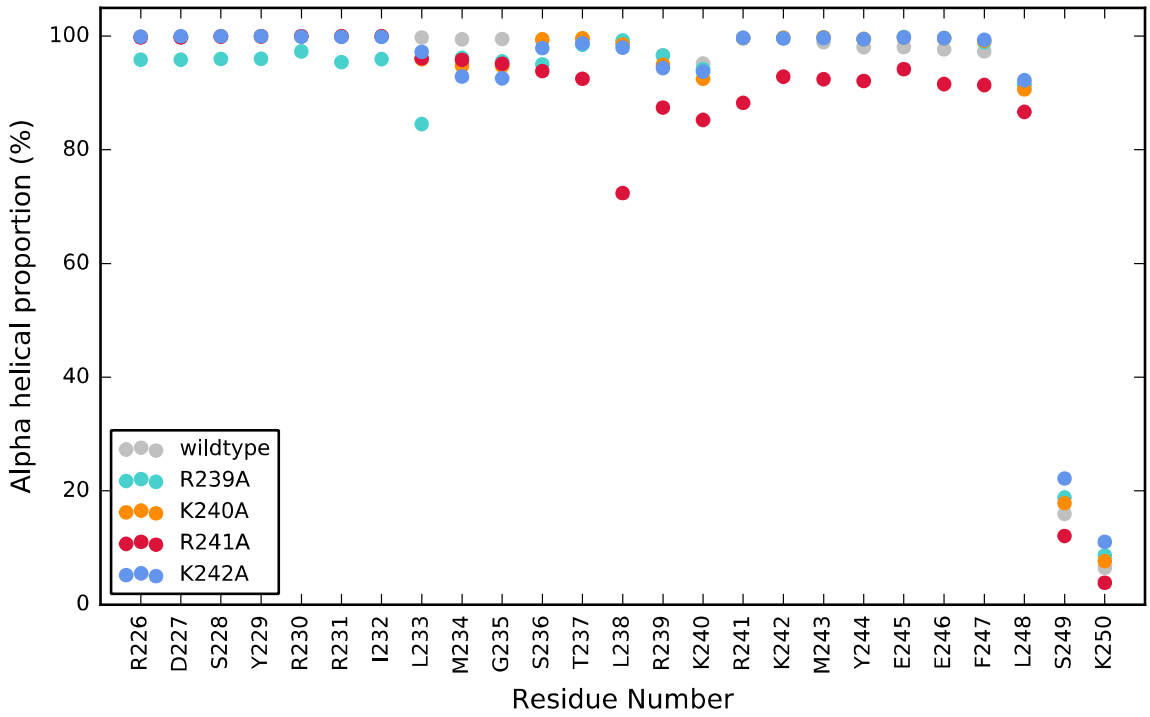


Figure S4. Per-residue analysis of helical proportion for residues located in the B/C helix.

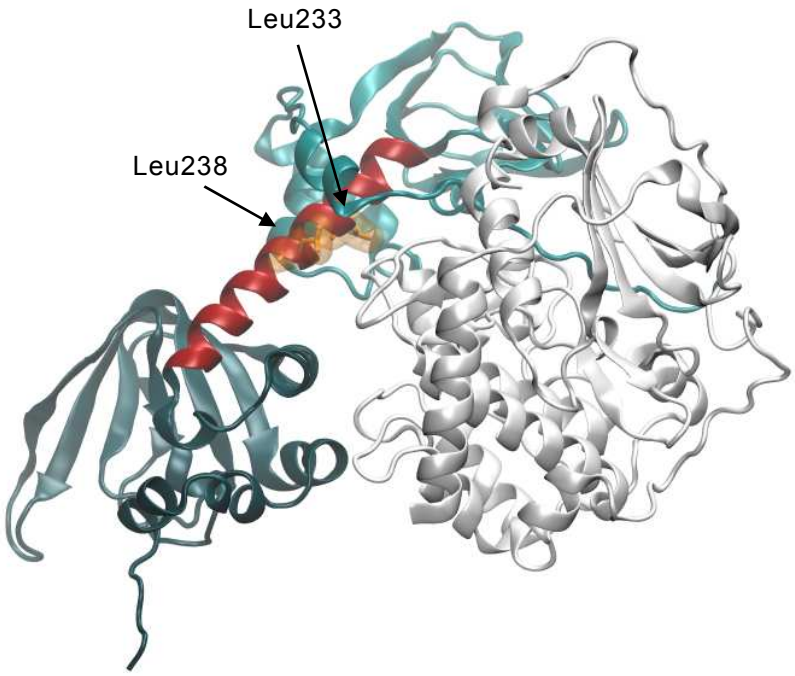


Figure S5. Representation of the two residues in the B/C helix with smallest helical proportion as verified from the simulations (Leu233 in R239A and Leu238 in R241A simulations, shown in orange) on the holoenzyme crystal structure.

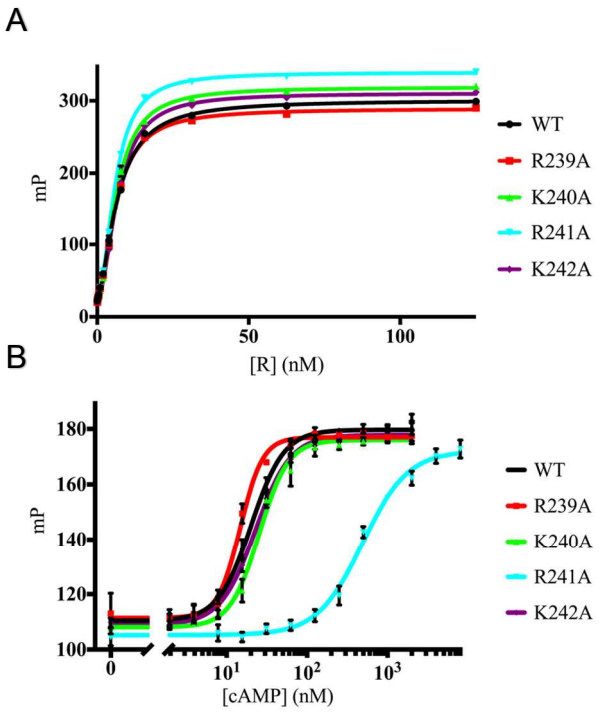


Figure S6. Nucleotide Binding and Allosteric Activation of RIα B/C Helix Basic Patch Mutants. (a) To assess cyclic nucleotide binding to RIα, the fluorescent cAMP analogue, 8-[fluo]-cAMP, was used to measure fluorescent polarization to R subunits titrated at various concentrations (0 nM – 125 nM). (b) To evaluate allosteric activation of RIα mutant holoenzymes, polarization of the fluorescent PKA inhibitory peptide, 5/6-FAM-IP20, by binding to dissociated C subunit was assessed in response to titrating concentrations of cAMP (0 nM – 8000 nM). (a-b)

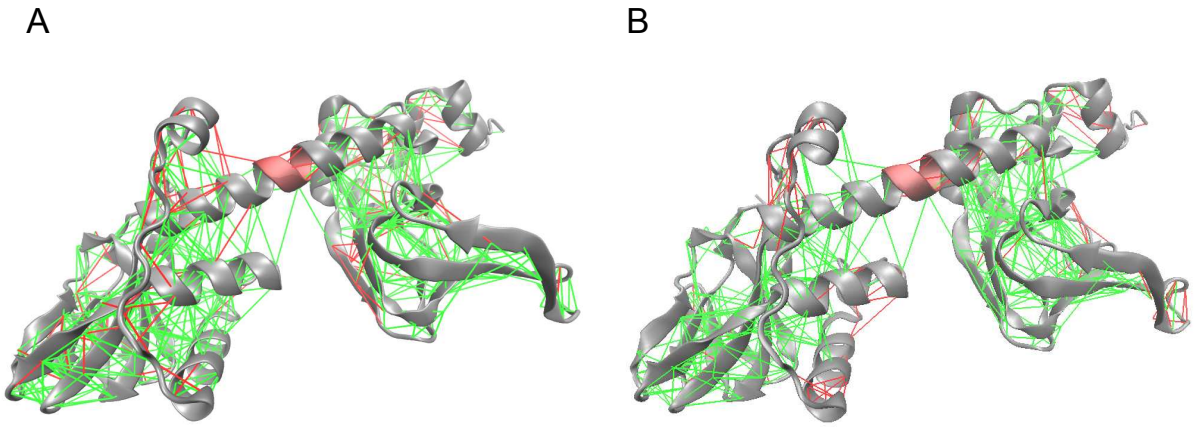


Figure S7. Analysis of protein frustration using the protein frustratometer methodology: (a) mutational frustration and (b) configurational frustration. Highly frustrated contacts are represented as red lines, neutral contacts as grey line and minimally frustrated contacts as green lines. The backbone of the residues in the basic patch of the B/C helix are highlited in red.

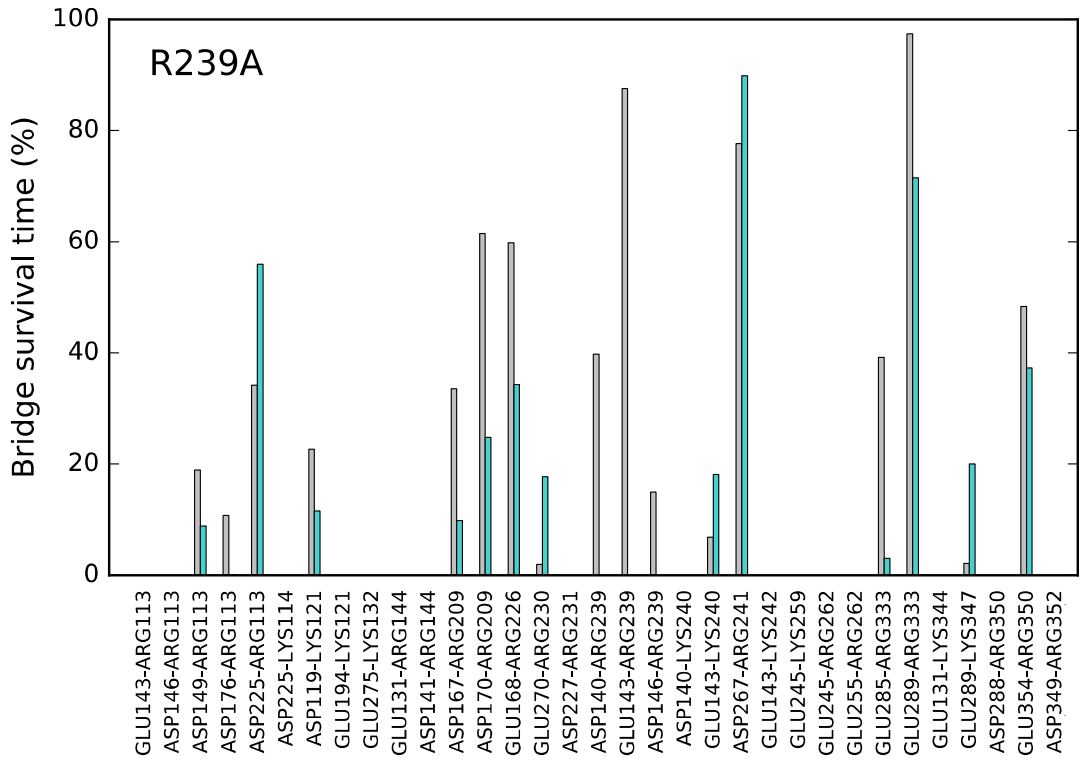


Figure S8. R239A salt bridges' lifetime (in turquoise) for those that were altered by more than 10% with the mutation, compared to wildtype (gray). The abolished salt bridges are highlighted.

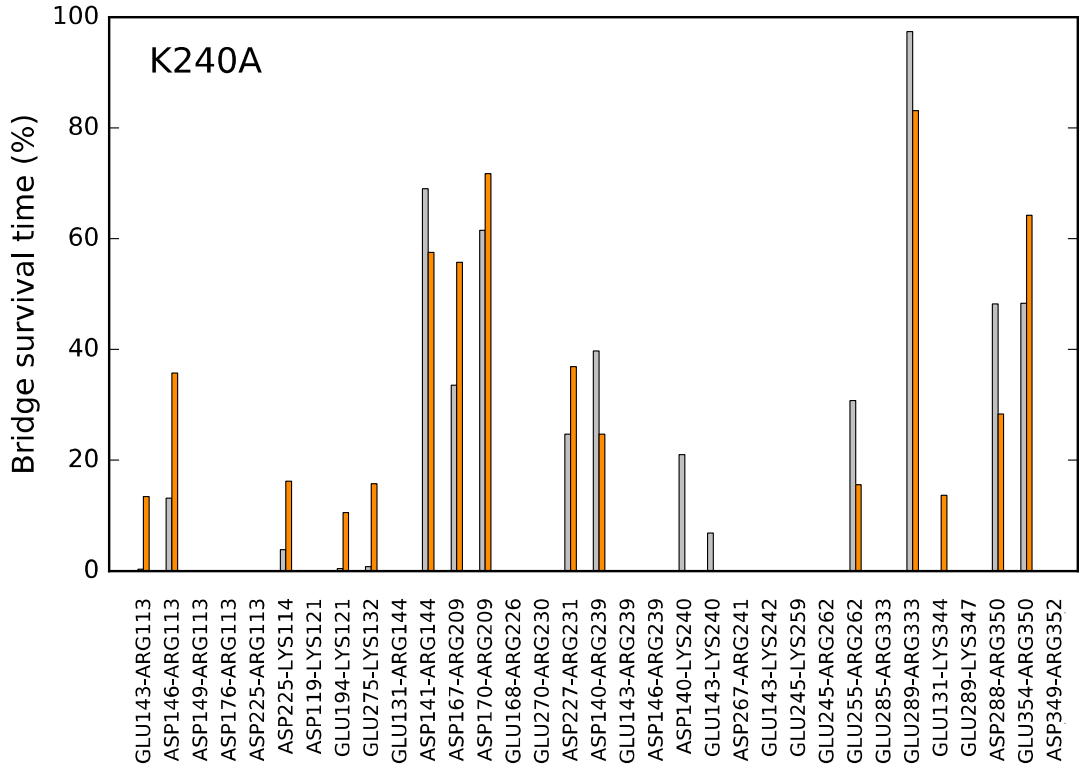


Figure S9. K240A salt bridges' lifetime (in orange) for those that were altered by more than 10% with the mutation, compared to wildtype (gray). The abolished salt bridges are highlighted.

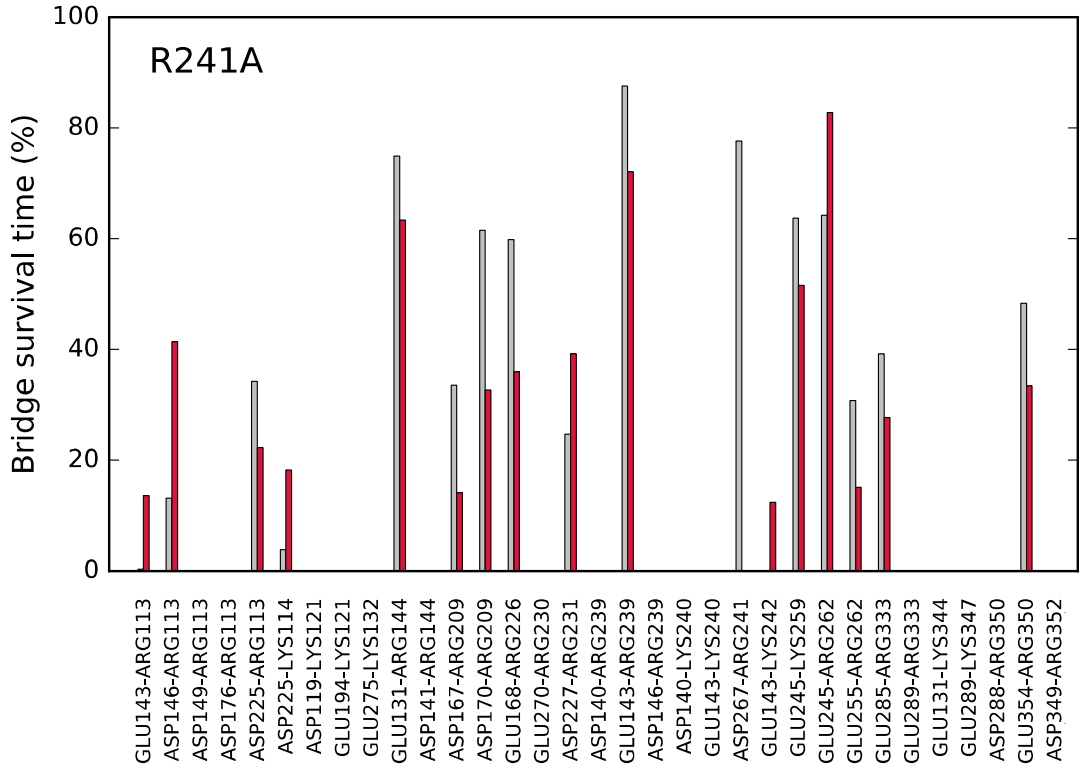


Figure S10. R239A salt bridges' lifetime (in red) for those that were altered by more than 10% with the mutation, compared to wildtype (gray). The abolished salt bridge is highlighted.

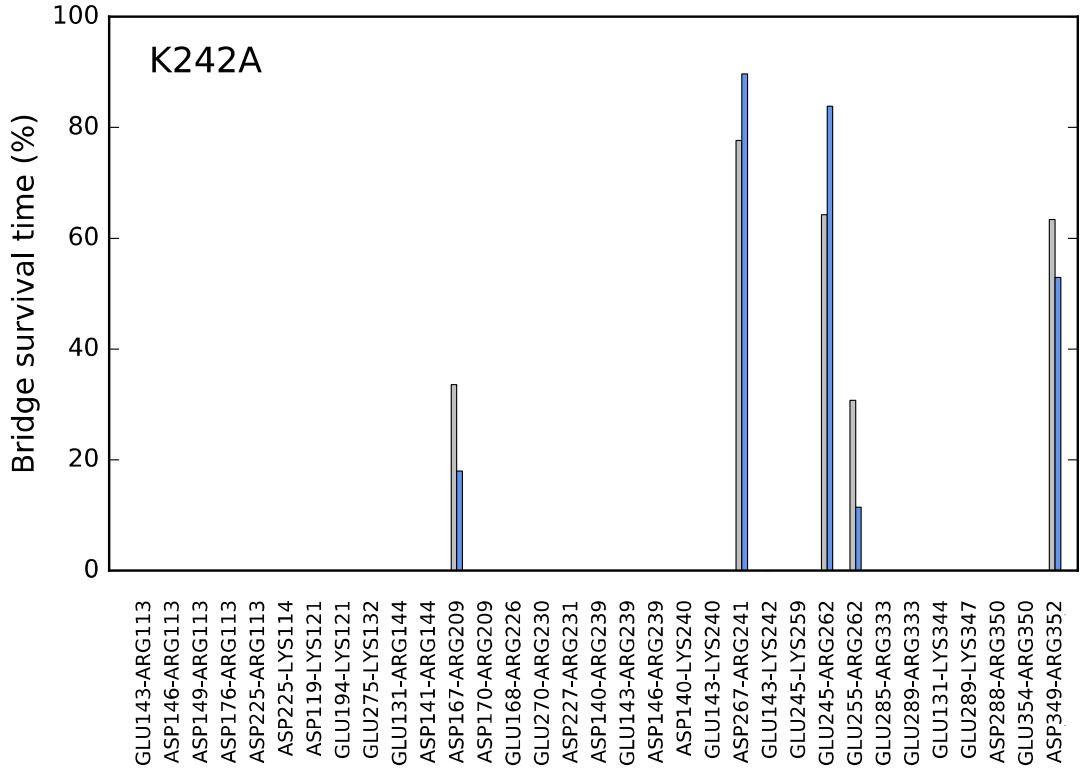


Figure S11. R239A salt bridges' lifetime (in blue) for those that were altered by more than 10% with the mutation, compared to wildtype (gray). The abolished salt bridge is highlighted.